[Background of the Invention and Prior Art Technologies]

As people's diet become more diverse than ever, "obesity" is one of the serious worries of modern day people. Obesity is not only undesirable from an aesthetic point of view, but also known to cause various disorders like diabetes, arteriosclerosis, hypertriacylglycerolmia, hypercholesterolemia and thrombosis.

[0003]

Obesity is caused by differentiation and enlargement of fat cells, or the increased number of the fat cell itself, and in any case "glucose uptake" is deeply involved with.

[0004]

Meanwhile, as glucose is a polar substance, it requires a transport carrier for uptake of glucose into each cell from blood (i.e., glucose transporter: GLUT). At present, nine types of GLUT (GLUT1 to 9) have been cloned, and among them, GLUT1 and GLUT4 are expressed in the fat cells that are greatly involved with a carbohydrate and lipid metabolism in a living body. Among them, GLUT4 particularly plays a key role for the glucose uptake activity on the membrane of fat cells.

[0005]

GLUT4 is called insulin-sensitive type GLUT and typically present in an intracellular vesicle in fat cells and muscle cells. When stimulated by insulin, it migrates (i.e., translocation) to a cell membrane and becomes in a

state ready for glucose uptake. The translocation of GLUT4 is initiated by a signal sue to binding of insulin to a receptor and autophosphorylating of a β subunit of the receptor, and according to a pathway including phosphorylation of an insulin receptor substrate (IRS), activation of phosphatidylinositol 3 kinase, activation of Akt/Protein Kinase B and exocytosis from the endoplasmic reticulum in the cell to the cell membrane, the migration is completed. Moreover, it has been reported that, GLUT4 has a function of delivering more glycogens to muscle cells and athletics have more GLUT4 than general people ("Most recent findings about carbohydrate loading", Bulletin by Sports Medicine Research Center of KEIO University, 1996). [0006]

In view of the above aspects, the inventors of the invention studied the effect of a tea extract and its components on glucose uptake activity and functions of GLUT4, etc., and as a result came up with many findings. Based on the results, the present invention was achieved. [0007]

Moreover, regarding the function of a tea extract, an agent for lowering cholesterol in blood plasma which contains polysaccharides included in tea leaves (i.e., ribose, Arabinose and glucose) as an effective component is disclosed in Japanese Patent Application Laid-Open (JP-A) No. 06-80580. It is disclosed in JP-A No. 10-158181 that the lipolysis promoter containing a plant extract extracted

from tea promotes reduction of adipose tissues in the whole or a local part of the body and has an effect on improvement of obesity tendency and prevention and inhibition of obesity. It is disclosed in JP-A No. 11-302168 that a glucose uptake inhibitor containing epicatechin gallate in a tea extract as an effective component inhibits the glucose uptake in intestinal tract, and therefore is effective for treatment of obesity or diabetes, etc.

[0008]

[Means for Solving the Problems]

The invention provides a glucose uptake inhibitor and an insulin stimulation-responsive glucose uptake inhibitor in fat cells containing any one of catechin gallate, catechin having a gallate ester and a tea extract as an effective component.

[0009]

By taking the effective component of the invention, glucose uptake in fat cells, in particular glucose uptake increased by insulin stimulation, is inhibited so that the amount of the fat cells can be lowered. As a result, an excess lipid state, i.e., obesity, and various disorders like diabetes, arteriosclerosis, hypertriacylglycerolmia, hypercholesterolemia and thrombosis that are caused by obesity can be prevented and treated. Moreover, the effective component of the invention not only inhibits glucose uptake in fat cells but also activates glucose

uptake in muscle cells so that excess glucose can be taken into the muscle cells and consumed. Thus, without increasing sugar concentration in the blood or having lassitude caused by obesity control, improvement of the activity can be obtained.

[0010]

Although there can be many mechanisms including inhibition of translocation of a glucose transport carrier present in fat cells or binding to an insulin receptor, etc. as a mechanism of inhibiting glucose uptake activity in fat cells, in the present study, specific inhibition of GLUT4 translocation by the effective component of the invention is elucidated. Thus, the invention provides a GLUT4 translocation inhibitor in fat cells containing any one of catechin gallate, catechin having a gallate ester and a tea extract as an effective component.

[0011]

Moreover, it is provided by the invention a glucose uptake activator in muscle cells comprising any one of catechin gallate, catechin having a gallate ester and a tea extract as an effective component. By taking the effective component, GLUT4 translocation in muscle cells is activated so that glucose uptake activity in the muscle cells is enhanced, and as a result, a great amount of energy source is introduced to the muscle cells and the amount of muscle cells can be increased. Thus, it can be effectively used for activation of muscle tissues, lowering physical

tiredness, enhancement of exercise performance, strengthening and enhancement of muscle tissues as well as improvement of body constitution, etc. As such, a GLUT4 translocation activator in muscle cells or a muscle activator having any one of catechin gallate, catechin having a gallate ester and a tea extract as an effective component can be also provided.

[0012]

As for the effective component of the invention, any one of catechin gallate, catechin having a gallate ester and a tea extract can be used. Preferably, catechin having a gallate ester, especially catechin gallate, is preferable in that an excellent effect is expected from all the inventions.

[0013]

According to the present study, it is confirmed that the effect can be obtained by taking the effective component of the invention either before or after insulin secretion. Therefore, the effect can be obtained by taking the effective component of the invention before, during or after taking a food. Moreover, as the effective component of the invention is a component originating from tea that can be confidently taken as being commonly enjoyed from old times, it can be taken for a long period of time with confidence and no trouble. As a result, it is particularly effective for basic treatment and prevention of chronic symptoms and disorders and also for improvement of body

constitution.

[0014]

In addition, the catechin in the invention indicates (-)-catechin, and when the description "(-)" is omitted, it still means (-)-catechin. For example, catechin gallate means (-)-catechin gallate.

[0015]

[Best Mode for Carrying out the Invention]

All of the "insulin stimulation-responsive glucose uptake inhibitor", "glucose uptake inhibitor in fat cells", "GLUT4 translocation inhibitor in fat cells", "glucose uptake activator in muscle cells", "GLUT4 translocation activator in muscle cells", and "muscle activator" can be produced by containing a "tea extract", "catechin having a gallate ester" or "catechin gallate" with an appropriate concentration.

[0016]

The "tea extract" is obtained by extraction of tea (tea leaves, tea shoots and tea trees, etc. of Camellia sinensis are included), and an extract of any one (i.e., single use) of raw tea leaves, fermented tea like black tea and puer tea, semi-fermented tea like oolong tea and Pouchong tea and non-fermented tea like green tea, roasted green tea and Hoji tea or a mixture of two or more, or a mixture of the extract obtained from each can be used. However, from the viewpoint of the effect of the invention, for example an inhibitory activity for the insulin

stimulation-responsive glucose uptake, Sen tea instead of Ban tea or Gyokuro among green teas, Nuwara tea instead of Dimbula tea or Uba tea among black tea, Ti Guan Yin tea and Sechung tea instead of Shui Hsien tea among oolong tea are preferably used.

[0017]

Moreover, examples of the "tea extract" include an extract obtained by extracting tea with water, hot water or boiling water, preferably hot water of 40°C to 100°C, preferably an extract obtained by extracting with hot water of 90 to 100°C, and more preferably a tea extract which is purified to increase the content of catechin, specifically catechin having a gallate ester, and more specifically (-)-catechin gallate by purification means like resin adsorption, filtration including ultrafiltration and reverse osmosis filtration, or distribution extraction using ethyl acetate, etc., or a tea extract concentrate which is obtained by concentration or drying of these tea extracts. Preferred examples of the green tea extract include the green tea concentrate which is obtained by hot-water extraction of green tea and drying the extract to have about 30% of catechin concentration (trade name: TEAFRAN 30A, manufactured by Ito En LTD.) or the green tea concentrate which is obtained by hot-water extraction of green tea, treating the extract according to a column method to exclude the components other than catechin and drying to have about 85 to 95% of the tea polyphenol

concentration (trade name: TEAFRAN 90S, manufactured by Ito En LTD.).

[0018]

Meanwhile, the "catechin having a gallate ester" includes any one of (-)-epicatechin gallate, (-)-epigallocatechin gallate, (-)-catechin gallate and (-)-gallocatechin gallate, a polymer of any one of them, a copolymer of at least two of them, and a mixture of at least two of them. Among them, those containing 25% or more of (-)-catechin gallate, and more preferably isolated "(-)-catechin gallate" are preferable. The "catechin having a gallate ester" is present, in addition to tea, in various plants like cola, rhubarb, apple, peach, pear, cocoa bean and dried persimmon, and it can be obtained by extracting these plants. It is known that a polymer or a copolymer of catechin is produced by retort sterilization of the catechin or its mixture.

[0019]

Each of the "tea extract", "catechin including a gallate ester" and "(-)-catechin gallate" can be mixed as a single effective component. However, it is also effective to mix them as an effective component with a substance that has been already or will be recognized with an activity of inhibiting insulin stimulation-responsive glucose uptake, an activity of inhibiting glucose uptake in fat cells, an activity of inhibiting GLUT4 translocation in fat cells, an activity of activating glucose uptake in muscle cells, an

activity of activating GLUT4 translocation in muscle cells or an activity of activating muscle. Moreover, when it is mixed as a single effective component, it can be provided as a pharmaceutical preparation in which each of the "tea extract", "catechin having a gallate ester" and "(-)-catechin gallate" is dissolved in purified water or physiological saline, etc. (for example, an orally administered preparation, an intraperitoneally administered preparation and an intracerebrally administered preparation).

[0020]

All of the "insulin stimulation-responsive glucose uptake inhibitor", "glucose uptake inhibitor in fat cells", "GLUT4 translocation inhibitor in fat cells", "glucose uptake activator in muscle cells", "GLUT4 translocation activator in muscle cells" and "muscle activator" of the invention can be used as an orally administered preparation or a parenterally administered preparation (muscle injection, intravenous injection, subcutaneous administration, rectal administration, transdermal administration, and nasal administration, etc.), and it is preferably prepared in a mixture and a dosage form that is appropriate for each type of administration. With respect to the dosage form, for an orally administered preparation, it can be prepared in the form of a liquid, a tablet, powder, a granule, a sugar-coated tablet, a capsule, a suspension, an emulsion and a pill, for example. For a

parenterally administered preparation, it can be prepared in the form of an injection solution, an ampoule, a rectally administered preparation, an oil-based suppository and a water soluble suppository. With respect to the composition (i.e., preparation), it can be prepared according to a general method by using a vehicle, an extender, a binding agent, a wetting agent, a disintegrating agent, a surface active agent, a lubricating agent, a dispersant, a buffer agent, a preserving agent, a solubilization aid, a preservative, a flavor and fragrance, a soothing agent and a stabilizing agent, etc. Moreover, it is also possible to add non-toxic additives like lactose, fructose, glucose, starch, gelatin, magnesium carbonate, synthetic magnesium silicate, talc, magnesium stearate, methyl cellulose, carboxy methyl cellulose or its salt, gum Arabic, polyethylene glycol, syrup, vaseline, glycerin, ethanol, propylene glycol, citric acid, sodium chloride, sodium sulfite and sodium phosphate.

[0021]

Moreover, all of the "insulin stimulation-responsive glucose uptake inhibitor" , "glucose uptake inhibitor in fat cells", "GLUT4 translocation inhibitor in fat cells", "glucose uptake activator in muscle cells", "GLUT4 translocation activator in muscle cells" and "muscle activator" of the invention can be provided as, in addition to a pharmaceutical product, a non-pharmaceutical product, a health food, a health drink, a food product for special

health care and a functional food product having a pharmaceutical effect, and other pharmaceutical preparation or feed for animals other than human, etc. For example, after produced as a non-pharmaceutical product, it can be prepared in drinkable form like a bottled drink or the form including a tablet, a capsule and a granule to promote easier intake. As for the health food, health drink, food product for special health care and functional food having a pharmaceutical effect can be prepared by, for example, mixing the effective component of the invention with carbonic acid, a vehicle (including a granulating agent), a diluent, and also one or more selected from a food product group including various protein or carbohydrate substances like sweetening agent, flavor, wheat flour, starch, sugar, fats and oils, vitamins and minerals, or can be added to a presently known food product like sports drink, fruit drink, milk drink, tea drink, vegetable juice, dairy drink, alcohol drink, jelly, jelly drink, carbonate drink, chewing gum, chocolate, candy, biscuit, snack, bread, dairy product, processed fish product, animal product, ice candy, dried food product, and a supplement. Among these, the food product which is obtained by adding the "isolated catechin gallate" to a food substance containing a great amount of carbohydrates can be provided as a favorable food product for lowering fat (in other words, a diet food product) and a food product for muscle activation, etc.

[0022]

According to the invention, the content of the effective component may vary depending on the method of use. However, for a pharmaceutical product, it is preferably mixed in an amount of 0.001% to 1% by weight, in particular 0.01% to 0.5% by weight in terms of the weight of dry catechin. For a food product, it is preferably mixed in an amount of 0.001% to 1% by weight, in particular 0.01% to 0.5% by weight in terms of the weight of dry catechin. When it is prepared as a food product for lowering fat or a food product for muscle activation, it is preferable that 0.001% to 1% by weight of the isolated catechin gallate is mixed in a food product so that the concentration of catechin gallate is 5 to 500 times the concentration in tea that is usually consumed. Moreover, the intake dosage is about 10 mg to 5000 mg, and preferably about 100 mg to 1500 mg per day in terms of the weight of dry catechin.

[0023]

<Test 1>

According to the present test, a change in blood components and glucose uptake in adipose tissues and muscle tissues were examined to determine any change in carbohydrate metabolism and lipid metabolism when a rat had free access to a green tea extract.

[0024]

(Rat breeding)

For the test, a male Wistar rat right after weaning (three-week old) was used. The rats were divided into two

groups (5 animals per each group), and one group had free access to a green tea extract (trade name: OI OCHA, manufactured by Ito En, Ltd.) and the other group as control had free access to ion exchange water for three weeks. Body weight, food intake amount and water intake amount were measured everyday. Three weeks later, the rat that has been fasted for four hours was anaesthetized. Blood was taken from the heart and after laparotomy the adipose tissues and the femoral muscle were removed and used for the test.

[0025]

(Measurement of blood plasma components)

The blood taken from the above was separated into hemocytes and blood plasma by centrifuge. Using the blood plasma obtained, the blood sugar level, amount of triacylglycerol, amount of free fatty acids, total amount of cholesterol and amount of HDL cholesterol were measured using a measurement kit manufactured by Wako Pure Chemicals Industries.

[0026]

(3-O-Methyl-D-glucose (herein below, referred to as "3-OMG") uptake in adipose tissues and muscle tissues)

The adipose tissues and the muscle tissues of femoral region were removed from the rat and chopped finely. The tiny tissue strip (about 100 mg) was incubated in Krebs-Ringer-Hepes buffer (KRH) containing 5.5 mM glucose for 80 minutes. The buffer was replaced with glucose-free KRH and

the incubation was carried out for 15 minutes. Then, the uptake of 3-OMG (6.5 mM, 0.5 μCi) labeled with ^{3}H was allowed for 30 seconds. After that, by using the KRH solution containing 0.3 mM phloretin, the uptake was terminated. After washing with the same solution for several times, complete lysis was carried out by using NCSII solubilizer. After the lysis, the uptake amount of 3-OMG in the tissues was measured by using a liquid scintillation counter.

[0027]

<Test 2>

To examine at cellular level the influence of the tea components on glucose transport mechanism, cells of 3T3-L1 cell line, i.e., a precursor fat cell originating from a mouse, were differentiated into fat cells. After adding the "tea extract" or "catechin" to the resulting cells, glucose uptake into the cells and GLUT4 translocation were determined.

[0028]

(Preparation of tea extract)

As a "tea extract" , total nine kinds of the extract obtained from green tea (Sen tea (product from Honyama) · Gyokuro (product from Asahina) · Ban tea (product from Shizuoka)), black tea (Nuwara · Uba · Dimbula) and oolong tea (Ti Guan Yin · Sechung · Shui Hsien), i.e., three kinds for each tea type, were used for the test. In addition, each of the tea extraction was carried out by adding about

200 mL of hot water (about 90°C) to 10 g of the tea leaves, extracting for 10 minutes under proper stirring, and concentrating the extract using an evaporator followed by freeze-drying.

[0029]

For the glucose uptake test, those prepared by suspending in KRH to 5 mg/mL were used. Moreover, for the GLUT4 translocation test, those prepared by suspending in phosphate buffered saline (PBS) to the same concentration as the uptake test were used. Meanwhile, as a "catechin", total eight kinds including catechin (C), epicatechin (EC), gallocatechin (GC), epigallocatechin (EGC), catechin gallate (Cg), epicatechin gallate (ECg), gallocatechin gallate (GCg) and epigallocatechin gallate (EGCg) were used. These were dissolved in dimethyl sulfoxide (DMSO) to have 10 mM and used for the uptake test and the GLUT4 translocation test.

[0030]

(Culturing of the precursor fat cell line 3T3-L1 and induction of differentiation into fat cells)

3T3-L1 cells, i.e., a precursor fat cell originating from a mouse, were added to a 35 mm- or 100 mm-dish and cultured until the confluency, and then induction of differentiation into fat cells was performed. The induction of differentiation was carried out by culturing the cells for 3 days in DMEM medium (containing 10% fetal bovine serum (FBS)) added with 1 μM dexamethazone, 0.5 mM

3-isobuthyl-1-methylxantine, 10 μg/mL insulin and 100 μM ascorbic acid phosphate. Subsequently, the cells were cultured for two days in DMEM containing 10 μg/mL insulin and 100 μM ascorbic acid phosphate. After that, the culture was continued for 5 to 8 days in DMEM medium containing 10% FBS, and the cells that are found out by measurement using a microscope to be in the stage at which about 90% of the cells are differentiated into fat cells were used for each test.

[0031]

(Uptake of 3-OMG by the cells)

After adding 3T3-L1 cells to a 35 mm dish and allowing them to differentiate, the cells were cultured in advance in a serum free medium for 18 hours for desensitization. The influence of the tea extract and catechin was determined by using the two methods described below. As a first method, the tea extract (final concentration 100 μg/mL) or catechin (50 μM) was added to the cells before adding insulin and maintained for 15 minutes. Thereafter, 100 nM of insulin was applied for 15 minutes, and then the uptake of ^{3}H-labeled 3-OMG (6.5 mM, 0.5 μCi) was allowed for 30 seconds. As a second method, 100 nM of insulin was applied in advance to the cells for 15 minutes to have the translocation of GLUT4, and by adding the tea extract the uptake of ^{3}H-3-OMG was allowed. In both methods, the uptake of ^{3}H-3-OMG by the cells was limited to 30 seconds and the reaction was terminated by

rapid washing of the cells with the KRH solution containing 0.3 mM phloretin, which is an inhibitor for GLUT. Again, the cells were washed three times with the KRH solution containing 0.3 mM phloretin, lysed by the 0.5% aqueous SDS solution, and the uptake value of ^{3}H by the cells was measured by using a liquid scintillation counter. As a blank, the cells in which the non-specific adsorption of ^{3}H-3-OMG to the cells is inhibited in advance by phloretin were measured in the same manner. The value obtained by subtracting the blank from the uptake value of ^{3}H by the cells was defined as a true glucose uptake amount.

[0032]

(Preparation of cell membrane and detection of GLUT4 by Western blot analysis)

For the detection of GLUT4 by Western blot analysis, 3T3-L1 fat cells which had been seeded and differentiated in 100 mm dish were desensitized in the same manner as above, treated for 15 minutes with the tea extract (i.e., the tea extract described before, i.e., the tea extract obtained by adding about 200 mL of hot water (about 90°C) to 10 g of the tea leaves, extracting for 10 minutes under proper stirring, and concentrating the extract using an evaporator followed by freeze-drying, was applied in an amount of 50 μg/mL), stimulated with insulin for 15 minutes, and then used. The cells were homogenized and subjected to density gradient ultracentrifuge to prepare a cell membrane fraction. Protein amount in thus obtained cell

membrane fraction was determined and 1 μg of the same was subjected to SDS-PAGE to separate the proteins. The proteins after the separation was transferred to a PVDF (polyvinylidene difluoride) membrane and blocked by using Tris-buffered saline-Tween solution (TBST: 20 mM Tris-HCl (pH 8.0), 0.15 M NaCl and 0.05% Tween20) containing 5% skim milk. After washing several times with TBST, the membrane was reacted with an anti-GLUT4 antibody as a primary antibody and an anti-goat IgG antibody labeled with horseradish peroxidase as a secondary antibody. The immunocomplex present on the membrane was reacted with ECL plus chemiluminescence reagent, and according to the exposure of an X-ray film, GLUT4 was detected.

[0033]

(Detection of the phosphorylation of insulin receptor based on immunoprecipitation method)

For the determination of influence of the tea extract on phosphorylation of an insulin receptor (IR), 3T3-L1 fat cells which had been obtained by the same treatment as above (i.e., the cells that had been seeded and differentiated in 100 mm dish were treated for 15 minutes with the tea extract (i.e., the tea extract described before, i.e., the tea extract obtained by adding about 200 mL of hot water (about 90°C) to 10 g of the tea leaves, extracting for 10 minutes under proper stirring, and concentrating the extract using an evaporator followed by freeze-drying, was applied in an amount of 50 μg/mL), and

subsequently stimulated with insulin for 15 minutes were used. The cells were lysed by RIPA buffer (50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1% NP40, 0.5% deoxycholate and 0.1% SDS) to obtain the total extract. Protein amount in thus obtained extract was measured, and the protein of 1 mg portion was immunoprecipitated by an anti-IR-β antibody and the IR-β subunit which binds to the antibody was adsorbed on the protein A/G agarose. With centrifuge, the IR-β subunit bound to the antibody was recovered and subjected to SDS-PAGE to separate the proteins. After that, it was subjected to the Western blot analysis using an anti-phosphotyrosine antibody as a primary antibody. The Western blot analysis was performed in the same order as described above, and an anti-rabbit IgG labeled with horseradish peroxidase was used as a secondary antibody.

[0034]

<Results and analysis>

(With respect to the influence of green tea on the body fat of a rat)

To determine the influence of the tea extract at individual animal level, the rat was allowed to have free access to green tea for three weeks, and then a change in blood components and a glucose uptake activity in adipose tissues and muscle tissues were examined. Consequently, the following results were obtained.

[0035]

[Table 1]

	Control	Green tea
Body weight (g) final	146.8±6.3	144.4±13.1
Food intake (g) average	17.0±4.6	16.0±4.3
Water intake (tea) (mL) average	21.2±6.0	22.2±6.8
Tissue	Ratio in the body weight (%)	
Fat	2.68±0.06	2.35±0.11*
Liver	7.96±0.27	7.42±0.68
Spleen	0.54±0.03	0.53±0.04
Thymus gland	0.14±0.02	0.39±0.09
Kidney	1.42±0.33	1.34±0.09

Values are means±S.D.(n=5)

*Significant difference from control group.

($p<0.05$, by Student's t-test)

[0036]

[Table 2]

	Control	Green tea
Triacyl glycerol (mg/dl)	115.2±4.3	100.8±8.2
NEFA (meq/dl)	2.9±0.3	1.9±0.2*
Total cholesterol (mg/dl)	105.0±1.0	84.0±2.0*
HDL cholesterol (mg/dl)	51.5±1.6	42.0±1.9*
LDL cholesterol (mg/dl)	20.5±1.2	14.5±1.2*
Leptin (ng/ml)	8.8±0.5	7.7±0.5
Glucose (mg/dl)	196.0±4.5	204.0±7.6

Values are means±S.D.(n=5)

*Significant difference from control group.

($p<0.05$, by Student's t-test)

[0037]

[Table 3]

	^{3}H-3-OMG uptake (nmol/100 mg tissue)	
	Control	Green tea
Fat	16.2±2.6	13.9±1.7
Muscle	22.8±1.5	27.6±1.4*

Values are means±S.D.(n=5)

*Significant difference from control group.

($p<0.05$, by Student's t-test)

[0038]

(Change in the weight of rat adipose tissues according to the tea intake)

When the weight change in the rat group which had the tea extract was compared to the weight change in the control group which had ion exchange water only, no significant difference was recognized during the breeding period or right before sacrificing the animal (FIG. 1, Table 1). Moreover, no change in the food intake amount and the water intake amount was recognized either between the two groups (FIG. 2, Table 1). After sacrificing the animal, tissues were weighed. Although no significant difference was recognized, the weight of the adipose tissues was reduced by about 15%. Meanwhile, no change was recognized for the weight of a liver, a spleen, a thymus gland and a kidney. Moreover, according to the naked-eye observation, no abnormality was recognized for any organs including the above. From these results, it was determined

that the body weight of the rat did not change according to the intake of a tea extract and an effect of losing the body weight is not recognized for an animal in normal growth period. However, there is a tendency that the weight of the adipose tissues is reduced. This result suggests the possibility of the tea extract to inhibit the induction of differentiation from precursor fat cells to fat cells and the possibility of reducing the fat accumulation amount in the fat cells after the differentiation.

[0039]

(Change in the blood plasma components according to the tea intake)

The blood plasma components separated from the rat were examined. No change in blood sugar level was recognized according to the intake of the tea extract. Meanwhile, according to the intake of the tea extract, the amount of triacylglycerol was reduced by about 15% compared to the control group (Table 2). Meanwhile, there was a significant decrease that the amount of free acid was reduced by 35% and the total amount of cholesterol, the amount of HDL cholesterol and the amount of LDL cholesterol were all reduced by 20% or so. These results suggest that the components of the tea extract have an influence on lipid metabolism in a living body. Considering that at least the main organ and tissue that are responsible for the metabolism of triacylglycerol and cholesterol are a

liver, adipose tissues and a small intestine, it is evident that in these organs and tissues the tea extract regulates the system for absorption or synthesis and degradation of lipid components.

[0040]

(Effect of tea on the glucose uptake in adipose tissues and muscle tissues)

As a result of examining a change in ^{3}H-3-OMG uptake in the adipose tissues and muscle tissues, it was found for the rats of the tea intake group that ^{3}H-3-OMG uptake in the adipose tissues appeared to decrease while the uptake in the muscle cells significantly increased (Table 3). It is considered that the reduced glucose uptake in the adipose tissues in the tea intake group is closely related to the pattern of reduction in lipid weight (Table 1). Moreover, based on the result that no increase in the blood sugar level was recognized as shown in Table 2, it is believed that the glucose which was not taken into the adipose tissues is actively taken by muscles or other tissues.

[0041]

(Influence of the tea extract on glucose transport in 3T3-L1 fat cells)

From the results above, it was found that the total weight of the adipose tissues is reduced in accordance with the intake of the tea extract and the lipid metabolism of a living body is influenced thereby. Meanwhile, the adipose

tissues are responsible for the biosynthesis of a lipid from a carbohydrate. Thus, the influence of the tea components on the carbohydrate transport mechanism in the adipose tissues was determined at cellular level by using 3T3-L1 fat cell line. Consequently, the following results were obtained.

[0042]

(Influence of the nine kinds of tea extract on glucose uptake)

3-OMG uptake in the adipose tissues was increased by about 3 times according to insulin stimulation, and therefore it was confirmed that an insulin-responsive glucose transport mechanism is present in those cells. When the tea extract (100 μg/mL) was applied 15 minutes before insulin, all of the nine kinds of the tea extract used in this test lowered the 3-OMG uptake activity in the presence of insulin (FIG. 3). When the 3-OMG uptake was also measured in the absence of insulin, oolong tea was found to activate the uptake. Among the tea extracts which showed high inhibitory effect in the presence of insulin, Sen tea was selected and the concentration dependency of the inhibitory effect was examined (FIG. 4). When added before insulin, the extract of Sen tea reduced the uptake in a concentration dependent manner, and this effect was also recognized at a low concentration of 2 μg/mL. Next, using the cells to which the insulin stimulation had been applied in advance, the influence of the Sen tea extract on

the 3-OMG uptake was examined. The Sen tea extract (100 μg/mL) rapidly reduced the uptake over time till the treatment time of 1 minute (left column in FIG. 5). In addition, when the concentration dependency was determined at the treatment time of 1 minute, it was found that the uptake was reduced at the concentration of 5 μg/mL or more (right column in FIG. 5). Based on the results above, it is evident that the tea extract has an effect of inhibiting 3-OMG uptake when it is added either before or after insulin stimulation.

[0043]

(Influence of tea extract on the GLUT4 translocation)

In fat cells, the translocation of GLUT4 occurs according to insulin stimulation, and as a result the uptake amount of glucose increases. As such, it is expected that the tea extract has an activity for the signal transduction pathway of this translocation. Accordingly, the influence of the tea extract on the GLUT4 translocation and the influence of the tea extract on the phosphorylation of an IR-β subunit, which is the most upstream part, were examined. As a result of examining the GLUT4 translocation, insulin has remarkably increased the amount of GLUT4 protein on cell membrane. When three kinds of the green tea extract are applied before insulin stimulation, the GLUT4 translocation to the cell membrane caused by insulin was inhibited (FIG. 6). The inhibitory effect of Sen tea and Gyokuro was stronger than that of Ban

tea. In addition, Nuwara among black teas and Sechung and Shui Hsien among oolong teas showed the same inhibitory effect. Dimbula and Uba among black teas and Ti Guan Yin among oolong teas showed almost no influence on the translocation. Next, the influence of Sen tea extract on the phosphorylation of an IR-β subunit was examined by immunoprecipitation. As a result, the cells treated with Sen tea showed the phosphorylation of an IR-β subunit by insulin, which is the same as the control cells (FIG. 7). Moreover, the cells treated with Sen tea showed the same amount of IR-β subunit itself as the control cells and no change was recognized therefor. Moreover, when the phosphorylation of a tyrosine residue was examined by using the whole cellular proteins, it was found that the band near 95 kDa, which corresponds to the molecular weight of an IR-β subunit, is phosphorylated by insulin. However, a difference in the degree of phosphorylation due to the Sen tea extract was not recognized. From these results, it was learned that the Sen tea extract does not change the phosphorylation of an IR. Therefore, it is demonstrated that the tea extract specifically inhibits the GLUT4 translocation without affecting the binding of insulin to a receptor, i.e., the tea extract regulates the glucose transport without expressing an early signal for insulin.

[0044]

(Influence of catechin on the glucose uptake)

Viewing from the fact that the pattern of the result

varies depending on the tea type when the influence of a tea extract on glucose uptake is compared to the influence on the GLUT4 translocation, it was considered that the difference in composition or content of the components like catechin that are included in each extract is related to a behavioral change in the glucose transport mechanism. Accordingly, the influence of the 8 kinds of catechin on ^{3}H-3-OMG uptake was examined, and the following results were obtained. Among the catechins, four kinds of catechin (Cg, ECg, GCg, EGCg) having a gallate ester reduced the uptake amount of glucose, while remaining four kinds (C, EC, GC, EGC) did not reduce the uptake in the presence of insulin (FIG. 8). Among the four kinds with recognized inhibitory effect, the effect by Cg was the strongest. Based on these results, it was indicated that catechin in the tea extract, particularly catechin having a gallate ester, and more particularly catechin gallate (Cg) among them has an effect on the glucose transport mechanism of 3T3-L1 fat cells. In addition, since theaflavin as a polymer is abundantly contained in a black tea, the 3-OMG uptake was examined for the theaflavin mixture. As a result, quite strong inhibitory effect was recognized therefor.

[0045]

<Summary>

When the rat was allowed to intake the tea extract, it was indicated that its body weight did not change but

the weight of adipose tissues tended to decrease. As the adipose tissues are the place in which biosynthesis of a lipid from a carbohydrate occurs, if the glucose transport activity is lowered or a lipolysis activity by a lipoprotein lipase or a hormone sensitive lipase, etc. is increased, a decrease in the weight of adipose tissues is caused. As the fat accumulation is related to an occurrence of lifestyle disorders like diabetes, the intake of a tea extract is expected to have an effect of preventing lifestyle disorders. Moreover, since a normal rat right after weaning (three-week old) was allowed to intake the tea, it is highly possible that the decreasing tendency of the adipose tissue weight is caused by the inhibited differentiation of fat cells based on the inhibited glucose transport activity. Viewing from the result that the tea extract inhibited the glucose transport activity in a cell culture system in the presence of insulin, it is believed that in a state in which blood sugar level increased according to the intake of food and insulin from pancreatic β cells also increased the tea extract inhibits the carbohydrate transport into adipose tissues to prevent supply of excess energy. Meanwhile, although it was expected that high blood sugar level is caused by this inhibitory effect, a change in blood sugar level was not recognized for the rats which have had the tea. Instead, the glucose uptake in the muscle tissues was increased. From this result, it is believed that the tea

diverts excess energy to a locomotive organ and contributes to the increased energy. This implies that, although both of the muscles and adipose tissues are differentiated from an endoderm and both express GLUT4, due to a tea extract, isoforms of the same GLUT exhibit completely contrary behavioral pattern in different organs and tissues. Moreover, as an effective component which gives the effects described above, it was recognized that catechin in a tea extract, particularly catechin having a gallate ester, and more particularly catechin gallate among them shows a strong effect.

[0046]

(Example 1)

Following the prescription described below, a tablet was produced to obtain any one of the glucose uptake inhibitor in fat cells, insulin stimulation-responsive glucose uptake inhibitor, GLUT4 translocation inhibitor in fat cells, glucose uptake activator in muscle cells, GLUT4 translocation activator in muscle cells and muscle activator.

[0047]

Tea extract (TEAFRAN 90S or TEAFRAN 30A) … 120 mg

Vitamin C … 50 mg

Emulsified oligo carbohydrate … 90 mg

Granulating agent … 60 mg

Crystalline cellulose … 80 mg

Hydrogenated maltose syrup … 90 mg

Sucrose … 100 mg

Flavor … an appropriate amount

[0048]

(Example 2)

Following the prescription described below, a drink as a food and drink for reducing fats or a food and drink for activating muscles was produced.

[0049]

Catechin gallate … 50 mg

Vitamin C … 50 mg

Fructose and glucose liquid … 10 g

Water soluble dietary fiber … 500 mg

Flavor … an appropriate amount

Ion exchange water … 100 mL

[Brief Description of the Drawings]

FIG. 1 is a graph showing a change in body weight of a rat over time from the start of the intake of a tea extract according to Test 1.

FIG. 2 is a graph showing a change in food intake amount and water intake amount in a rat over time according to Test 1.

FIG. 3 is a graph showing the 3-OMG uptake activity in fat cells according to Test 2 wherein the treatment with various kinds of a tea extract was performed.

FIG. 4 is a graph showing the 3-OMG uptake activity in fat cells according to Test 2 wherein the treatment with a green tea extract was carried out before insulin

stimulation.

FIG. 5 is a graph showing the 3-OMG uptake activity in fat cells according to Test 2 wherein the treatment with a green tea extract was carried out after insulin stimulation.

FIG. 6 is a X-ray film photograph showing the effect of a green tea extract on GLUT4 translocation according to Test 2.

FIG. 7 is a X-ray film photograph showing the effect of a green tea extract on phosphorylation of an insulin receptor (IR) according to Test 2.

FIG. 8 is a graph showing the 3-OMG uptake activity in fat cells according to Test 2 wherein a treatment with various catechins was performed.